Integrating Life Stage Susceptibility into Immunotoxicity and Microbial Risk Assessment

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Integrating Immunotoxicological and Microbial Risk Assessment for Susceptible Life Stages
Regulatory Interest in Life Stage and Susceptibility

- “Pesticides in the diets of infants and children” (NRC, 1993)
  - Need for data on the effects of pesticides on the developing reproductive, immune, and central nervous systems.

- Food Quality Protection Act (EPA, 1996)
  - Separate assessment for infants and children to establish pesticide residue levels

- Safe Drinking Water Act (EPA, 1996)
  - EPA to conduct studies to identify sensitive subgroups

- Executive Order # 13045, 1997
  - Identification of potential health risks to kids a high priority
Agency Interest in Life Stage and Susceptibility

- ORD Human Health Multiyear Research Plan, Long-Term Goal 3: Susceptible Subpopulations -
  - Is there differential life-stage responsiveness or exposure to environmental agents
  - What are the long-term effects of developmental exposure to chemicals?
  - How does aging affect responsiveness to environmental chemicals?
Agency Interest in Life Stage and Susceptibility

- ILSI/HESI Technical Panel: Agricultural Chemical Safety Assessment
  - Enhanced F-1 component of the traditional two generation reproduction study
  - Includes developmental immunotoxicity and developmental neurotoxicity endpoints

- Microbial Contaminant Candidate List Workgroup
  - Takes life stage and susceptible groups into account in screening criteria
Current Practice

- Microbial Risk Assessment
  - Model Development
  - Protect the Population

- Life Stage in Risk Assessment
  - Uncertainty Factors
  - UF Adjustments to Protect Life Stages

- Immunotoxic Risk Assessment
  - Model Development
  - Protect the General Population

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Basics of Immunology
Bone Marrow: Source of Immune System Cells

- Proerythroblast
- Polychromatic erythroblast
- Erythrocytes
- Basophil
- Eosinophil
- Neutrophil
- Granulocytes
- Agranulocytes
- Leukocytes
- Progranulocyte
- Myeloblast
- Hemocytoblast
- Lymphoblast
- Monoblast
- Megakaryoblast
- Megakaryocyte
- Lymphocyte
- Monocyte
- Thrombocytes
Immune System Anatomy

Figure 1-7 Immunobiology. 6/e. (© Garland Science 2005)
**Basics of Immunology**

The Immune Response

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**Innate Immunity**
- Phylogenetically ancient
- Rapid (minutes – hours)
- No cell proliferation required
- Limited recognition
- Limited memory (?) mammals

**Adaptive (Acquired) Immunity**
- First appeared in jawed fishes
- Slow (days)
- Requires proliferation and differentiation
- Infinite array of specificities
- Long-lasting memory
Basics of Immunology

- The adaptive immune response to antigen
  - Recognition as foreign
  - Antigen processing and presentation
  - Gene transcription, mediator release, cellular proliferation and differentiation, effector protein synthesis
    - All steps must work properly or function will suffer
Basics of Immunology

- Cells of the adaptive immune system
  - B lymphocytes: differentiate into plasma cells, produce antibody
  - T lymphocytes: mediator production, cytotoxicity, regulation
    - CD4+ T helper cells, T regulatory cells
    - CD8+ Cytotoxic T cells, T suppressor cells
Host Factors Influencing Resistance to Infection

- Age
- Gender
- Genotype
- Nutritional status
- Life style choices
- Life events
  - Acute toxicity
    - Stress response in lab animals
Life Stage and Immunocompetence

- The developing immune system
  - Innate immunity
    - Neutrophils
      - Provide 1st line of defense
      - Phagocytosis and killing, particularly extracellular bacteria
      - Lower rate of production in the bone marrow (easily depleted)
      - One third to ½ the content of bactericidal proteins vs adults
    - Complement system
      - Lysis of Ab-coated cells
      - Opsonization of encapsulated bacteria
      - Neonates have 60-80% of adult levels
    - NK cells
      - Nonspecific killing of certain tumor cells and some infectious agents
      - One third the number as adults (cord blood)
      - Bind only 2/3 as many targets as adults
      - Of those that bind, killing is about half adult level
Life Stage and Immunocompetence

- The developing immune system
  - Adaptive immunity
    - Humoral immunity
      - High level of protection via maternal IgG that wanes with age (by ~60% at 3 months)
      - IgM and IgG levels reach 50% of adult levels by 7-12 months; IgA by 3-5 years
    - Cellular immunity
      - 90% of neonate T cells are naïve
      - “Allergic” phenotype of cytokine production is the default at birth; decreased resistance to intracellular bacteria
  - Resistance to infection
    - Reduced innate and adaptive function increases susceptibility to:
      - Encapsulated organisms: e.g., group B Streptococcus, Haemophilus
      - Intracellular organisms: e.g., Listeria
      - Viral infections, e.g., influenza
Life Stage and Immunocompetence

■ The aged immune system
  ■ Innate Immunity
    ■ Neutrophils
      ■ Normal numbers
      ■ Increased rate of apoptosis
      ■ Ingest fewer bacteria/cell; less adept at killing ingested organisms
  ■ Adaptive immunity
    ■ Humoral immunity
      ■ Fewer Ab-producing cells
      ■ Less Ab produced/cell
      ■ Lower quality Abs are produced
    ■ Cellular immunity
      ■ Generalized decrement in function on a per cell basis
      ■ Decreased ratio of naïve to memory T cells
  ■ Resistance to infection
    ■ Reduced innate and adaptive function increases susceptibility to:
      ■ Encapsulated organisms, e.g., group B Streptococcus, Haemophilus
      ■ Intracellular organisms, e.g., Listeria
      ■ Viral infections, e.g., influenza
Xenobiotic Exposure and Immunocompetence

Exposure

Suppression
Infection
Neoplasia

Immune System

Stimulation (modulation)

Allergy
Autoimmunity
Consequences of Xenobiotic Exposure on Immunocompetence

- “Chemical AIDS”... not
  - Severe immunosuppression associated with opportunistic infections
  - Uncommon at the population level
    - HIV/AIDS
    - Severe primary immunodeficiency
    - Bone marrow and organ transplant patients
    - T helper (CD4+) cells decreased to < 500/µl (normal=1500-2500)

- Most likely outcome: mild to moderate immunosuppression
  - Recovery expected when exposure ends
    - Immune system redundancy
    - Immune system self-renewing
      - Developmental effects may be an exception
Immune Mediated Resistance to Infection

- Extracellular organisms (antibody, phagocytic cells)
  - *Staphylococcus, Streptococcus, Escherichia*
  - Certain helminths and protozoa
- Intracellular organisms (phagocytic cells, T cells)
  - *Listeria, Mycobacterium*
  - Viruses
  - Certain helminths and protozoa
- Toxic products or constituents (antibodies)
  - Tetanus toxin
  - Diphtheria toxin
- Transformed (neoplastic) cells (cytotoxic T cells, NK cells)
Organism Factors Influencing Host Resistance

- **Dose of the organism**
  - Few: rapidly eliminated
  - Many: overwhelms innate and adaptive resistance
    - May cause colonization
    - May cause disease

- **Virulence factors**
  - Ability to evade detection or destruction
  - Production of toxins and adherence factors
  - Rapid growth
  - Very low infectious dose
    - Noroviruses
    - *Cryptosporidium* (certain strains)
    - *Giardia*
Immunotoxicology Hazard ID

- Research in government and academic labs
- Nomination for testing by NTP
- Observations in routine toxicity testing
  - Mass, cellularity, architecture of spleen and thymus
  - Abnormal hematology
**Immunotoxicity Hazard ID**

EPA Health Effects Test Guidelines: OPPTS 870.7800
Immunotoxicity (TSCA, FIFRA)

**Inject SRBC**
5 days

**Measure relative or absolute antibody concentration by ELISA**

**Serum samples**

4 days

**Single cell suspension**

**Count relative (per million) or absolute (per spleen) number of cells producing antibody**

**Optional Assays**
Phenotypic analysis (if TDAR negative)
NK cell activity

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Draft immunotoxicity risk assessment guidelines

- Suppression
- Functional deficits considered more significant than observations
- Dose response
- Recognition of age-related sensitivity/susceptibility
Life Stage Sensitivity to Immunotoxicants and Risk Assessment

- Developmental vs. adult exposure to xenobiotics (DES, DZP, Pb, TCDD, TBTO)
  - Can cause long-lasting (± lifetime) effects at doses that cause short-term immunotoxicity in adults (e.g., DES, ± DZP)
    - Testing adults will detect effects, but grossly underestimate the relative risk of gestational/neonatal exposure
  - Can cause immunotoxicity at lower doses in the young than in mature animals, and effects are somewhat persistent (DZP, Pb, TBTO)
    - Application of an uncertainty factor may provide protection, but would not predict persistence
  - Can cause long-lasting (± lifetime) effects in offspring at doses that do not affect immune function in adults (e.g., TCDD in rats)
    - Testing adults only would fail to detect immunotoxicity
Implications for Risk Assessment in Sensitive Groups

- Persistence of effects
  - Simple delayed maturation
  - Incomplete maturation with long term consequences
- Critical windows of developmental sensitivity
  - Immune system maturation in rodents and humans
## Comparative Ontogeny

<table>
<thead>
<tr>
<th>Event</th>
<th>Mouse (days) (% of term)</th>
<th>Human (weeks) (% of term)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal liver begins functioning as a hematopoiesis site</td>
<td>10.5 (50%)</td>
<td>6 (15%)</td>
</tr>
<tr>
<td>Appearance of T cells in fetal liver</td>
<td>14 (67%)</td>
<td>6-8 (15-20%)</td>
</tr>
<tr>
<td>Organogenesis of thymus begins</td>
<td>11 (52%)</td>
<td>6 (15%)</td>
</tr>
<tr>
<td>Secondary lymphoid organs begin to develop</td>
<td>10.5 (50%)</td>
<td>7 (18%)</td>
</tr>
<tr>
<td>Lymph nodes start to appear</td>
<td>10.5 (50%)</td>
<td>8-12 (20-30%)</td>
</tr>
<tr>
<td>Spleen develops</td>
<td>13 (62%)</td>
<td>10-14 (25-35%)</td>
</tr>
<tr>
<td>B cell lymphopoiesis begins in bone marrow</td>
<td>17 (81%)</td>
<td>12 (30%)</td>
</tr>
<tr>
<td>B lymphocytes detectable in blood</td>
<td>13 (62%)</td>
<td>12 (30%)</td>
</tr>
<tr>
<td>CD4+ and CD8+ T cells detectable in spleen</td>
<td>19 (91%)</td>
<td>14 (35%)</td>
</tr>
<tr>
<td>Thymus development completed</td>
<td>13 (62%)</td>
<td>15-16 (37-40%)</td>
</tr>
<tr>
<td>Bone marrow becomes the major site of hematopoiesis</td>
<td>17.5 (83%)</td>
<td>22 (55%)</td>
</tr>
<tr>
<td>T cell receptor expression in periphery</td>
<td>Early post-natal</td>
<td>23 (58%)</td>
</tr>
</tbody>
</table>
DIT and Hazard ID

A. “Conventional” DIT Protocol

- Exposure to dams
  (variable designs; usually acute sometime during gestation)

- Gestation

- Lactation

- Parturition

- Weaning

- Young adults 8-10 wks old

B. “Alternative” DIT Protocol

- Exposure to dams

- Gestation

- Lactation

- Parturition

- Weaning

- PND 10

- PND 21

- Immune test

C. Proposed DIT Protocol for “all” critical windows

- Exposure to dams
  (direct dosing, as needed)

- Gestation

- Lactation

- Parturition

- Weaning

- PND 45

- Optional Exposure

- Young adults 8-10 wks old

from M.P. Holsaple et al.
Implications for Risk Assessment

- In the young
  - Developmental exposure may result in persistent immunosuppression
  - Late gestational exposure in rodents mimics early to mid-gestation in humans

- In the aged
  - Stress affects resistance to infection
  - Chemicals?
    - Critical data gap
Advanced Age, Chemical Exposure and Host Resistance


Mice


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Altered Host Resistance in Humans

- First Nations (Inuit) in Canada
  - High level maternal exposure to chlorinated compounds in diet
  - Greater rate of inner ear infections in children
- Dutch school children
  - Grouped by PCB concentration in breast milk
  - Increased rates of inner ear infections at highest exposure levels
- Elderly populations
- Stressed populations
Issues to Consider

- “Normal” individuals are susceptible to infection
  - Host factors
  - Properties and dose of the infectious agent
- Susceptibility to infection in individuals exposed to xenobiotics
  - Host factors
  - Properties and dose of the xenobiotic and of the infectious agent
  - Detecting increased susceptibility at the population level is difficult
- Protecting the public health
  - Who do we protect?
    - If we protect the most susceptible, will we protect the entire population?
  - Can life stage immunotoxicity data augment microbial risk assessment?
Challenges to Incorporating Life Stage

- Into microbial risk assessment
  - Are the models sensitive to small changes at the population level?
  - Outbreaks captured well, but are changes in incidence or severity of common infections?
  - Are default assumption adequate predictors of life stage sensitivity?
  - Can current models incorporate developmental immunotoxicity data?
Challenges to Incorporating Life Stage

- **Into immunotoxicity risk assessment**
  - Extrapolation of infection severity data in animals vs. incidence AND severity data in humans
    - Animal data: young adults under ideal conditions
    - Human data: various age groups under uncontrolled conditions
  - Extrapolation of lymphocyte data from animals and humans
    - Animal data primarily from lymphoid organs (spleen and thymus)
    - Human data primarily from peripheral blood
      - Differential distribution of cell types
Emphasis on Microbial Risk Assessment

Clinical data
Epi data

Life Stage Considerations

Microbial Risk Assessment

Model Development

Protect the Population

Host-Pathogen Profile

Host characteristics
Pathogen-disease characteristics

Immunology Data
L.S. Immunotox Data
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Emphasis on Immunotox RA

Immunotoxicology Risk Assessment

Model Development

Protect the General Population (Protect Life Stages)

Adult data (dose response)

Mandated EPA Life Stage Considerations

(Developmental data) (Dose response) (Persistence)

Microbial Data
1) Clinical
2) Epidemiological

Protect the General Population (Protect Life Stages)
Potential for Improved Risk Assessment

Protect General Population and Susceptible Life Stages and Pops.

Scaling of Uncertainty Factors?

Microbial Risk Assessment Data

Immunotoxicology Risk Assessment Data

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